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Gender differences in renal growth and function after uninephrectomy in adult rats

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Background. It is known that compensatory renal growth (CRG) following unilateral nephrectomy (UNX) increases both the size of the kidney and its functional capacity; however, few studies have investigated whether differences in CRG exist between the sexes. Our study examined the sex-related differences in remnant kidney growth and function two months following UNX.

Methods. Adult male and female Wistar rats underwent either left UNX or sham operation and recovered for 8 to 10 weeks. Another group of female rats underwent ovariectomy (Ox), with vehicle, estrogen, or testosterone replacement: two-weeks postsurgery animals underwent UNX and recovered for 8 to 10 weeks. Metabolic studies, acute renal function studies [response to acute saline volume expansion (2 to 4% of body wt) or phosphate (Pi) infusions in thyroparathyroidectomized rats (to determine the transport maximum (TmPi)], and renal morphology were assessed at the end of the experimental period.

Results. Two months post-UNX, male remnant kidneys grew 114 ± 7% of their excised kidney weight (KW), whereas female remnant kidneys grew only 57 ± 4% ($P < 0.05$). There was a significant increase in the glomerular volume of male remnant kidneys (126.2 ± 13.4%, $P < 0.001$) compared with control kidney volume, whereas there was no change in glomerular volume in female remnant kidneys (20.2 ± 16.1%, P = NS). There was also glomerular and tubular damage in the male remnant kidneys, whereas female remnant kidneys were intact. Studies in Ox female rats supplemented with gonadal steroids determined that testosterone is the driving force for the enhanced remnant kidney growth and glomerular hypertrophy. Renal function studies determined that UNX males had significantly higher glomerular filtration rates (GFRs) than UNX females, although the GFR/single KW was not different between the sexes, indicating a proportional increase in GFR. Basal urinary sodium excretion and urine flow rates were significantly higher in anesthetized UNX rats than their sham-operated controls, and urinary sodium excretion and urine flow rates in UNX males were significantly higher than in UNX females. Both male and female UNX rats responded to volume expansion with an exaggerated initial sodium and urine excretion compared with their controls. Phosphate handling was not altered in UNX male rats; however, UNX female rats had increases in fractional Pi excretion that were associated with significant reductions in the maximum capacity for Pi reabsorption (2.10 ± 0.07 vs. 3.43 ± 0.24 μmol/ml GFR in female controls, $P < 0.0001$). This difference was also observed in Ox rats treated with estrogen and testosterone (2.31 ± 0.07 vs. 3.12 ± 0.11 μmol/ml GFR, $P < 0.0007$).

Conclusions. These findings indicate that sexual dimorphism exists in remnant kidney growth and function two months following UNX. Indeed, morphological abnormalities and impairment in renal phosphate handling are affected by gonadal steroids by two-months post-UNX. The fact that renal Pi transport was reduced in female but not male UNX rats may also have important implications during periods of high metabolic demand for phosphate in the female.

Key words: compensatory renal growth, kidney development, unilateral nephrectomy, testosterone, estrogen, phosphate transport, sexual dimorphism.

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The removal of a single kidney immediately stimulates the growth and function of the remaining (remnant) kidney. We have previously determined that there are differential mechanisms initiating the early compensatory renal growth (CRG) response with age [1–4] and between the sexes (abstract; Mulroney et al, *J Am Soc Nephrol* 4:776A, 1992). Indeed, although early CRG is growth hormone (GH)-dependent but is independent of changes in renal IGF-I gene expression in the adult male animal [1, 2], CRG in the prepubertal male and adult female animals is GH-independent but associated with significant increases in remnant kidney IGF-I gene expression and ligand binding (abstract; ibid) [3, 4]. Furthermore, the alternate growth pathways appear ultimately to govern whether the accelerated growth has a hyperplastic component (abstract; ibid) [4], and the mechanisms may be mediated by the different gonadal steroids. This elucidation of the early age- and gender-related differences in renal growth highlights the potential importance of development and sex on physiological and pathophysiological processes.

Although some long-term effects of renal ablation

have been reported in both human and animal studies, the issue of gender differences is not well understood. Clinical perspective studies have reported that many kidney donors have at least mild proteinuria [5–10], and some have reported a higher incidence of hypertension than normal [7, 8]. When male and female kidney donors were assessed separately, males had a higher rate of proteinuria and hematuria compared with single-kidney women, as well as increased renal disease [11]. Animal studies have usually reported an increased incidence of glomerulosclerosis (GS) and other anomalies, possibly because they represent accelerated aging models, and analysis can be performed easily. Morphological studies in rats have determined that there is a higher incidence of GS in rats undergoing unilateral nephrectomy (UNX) at a young age, compared with adults potentially due to the greater remnant kidney growth rate in the young [12, 13]. Gender studies have shown that the UNX female rat has reduced proteinuria compared with males [14]. The elevated proteinuria in the one-kidney male may reflect an exacerbation of the normal development of glomerular injury in the male rat [15]. These studies, however, were performed nine months post-UNX. Seminal work by Reckelhoff and Baylis has illustrated that the relative abundance of renal metalloproteases contributes to the age-related degeneration of the male kidney and is related to the presence of testosterone (TEST) [16]. These studies support the notion that UNX is associated with long-term degeneration of the remnant kidney, an action that appears to be dramatically less pronounced in the female animal. However, no studies have integrated the early development of changes in kidney morphology with renal function and determined the significance of gender on these parameters. Defining the early development of renal pathology and potential sex differences would help elucidate the mechanisms by which renal parenchymal and functional damage occurs. Thus, the purpose of these studies was to determine changes in renal function and morphology in male and female rats at an intermediate time (two-months) post-UNX.

METHODS

Unilateral nephrectomy

All procedures were performed in accordance with guidelines approved by the Georgetown Animal Care and Use Committee. Adult (12 to 14 weeks of age) male and female Wistar rats were anesthetized with ether and were weighed. An incision was made in the left flank, and the kidney was isolated and decapsulated (the adrenal gland remained intact), ligated with 4-0 silk suture, and excised. Sham-operated animals were prepared in the same manner, and the left kidney was manipulated, but not removed. The area was cleansed with an antimicrobial agent (Novason), and the flank incision was sutured closed. The left, excised kidney was used as a control for the remnant kidney growth. It was weighed, sliced longitudinally, and placed in 10% formalin for morphological analysis. The animals recovered rapidly and were allowed normal rat chow and water *ad libitum.*

A separate group of adult female rats underwent ovariectomy (Ox) by flank incisions and were supplemented with either cholesterol vehicle (VEH; 5 μg cholesterol in 0.1 ml saline, intraperitoneally, at 9 a.m. every other day), estrogen (EST; 17β-estradiol, 5 μg in 0.1 ml vehicle, at 9 a.m. every other day), or TEST (TEST proprionate, by 15 mm silastic implant, subcutaneously) two weeks prior to UNX. This type of hormone replacement strategy has been shown to be effective in the rat [17]. After two weeks, animals underwent left nephrectomy, as outlined previously.

At two-months post-UNX, animals were weighed and separated into three groups:

Group 1. Male (sham, $N = 6$; UNX, $N = 6$) and female (sham, $N = 5$; UNX, $N = 6$) rats were prepared for acute renal clearance experiments to determine the maximum capacity for phosphate reabsorption (method follows).

Group 2. Male (sham, $N = 5$; UNX, $N = 6$) and female (sham, $N = 5$; UNX, $N = 6$) rats were prepared for acute renal clearance experiments to determine their response to saline volume expansion (method follows).

Group 3. Female Ox rats given VEH ($N = 5$), EST ($N = 5$), or TEST ($N = 5$) were anesthetized and prepared for acute renal clearance experiments to determine the maximum capacity for phosphate reabsorption (method follows).

Group 4. A separate group of adult male and female rats underwent UNX, and remnant kidney growth was determined 48 hours later to illustrate initial remnant kidney growth between the sexes. The left, excised kidney was used as a control for the remnant kidney growth. No clearance or morphological studies were performed on this group.

General surgical preparation for acute renal clearance experiments

Animals from groups 1, 2, and 3 were anesthetized by an intraperitoneal injection of Inactin (80 to 100 mg/kg, intraperitoneally; Promonta, Hamburg, Germany). Body temperature was maintained at 37 ± 0.5°C via rectal probe using a servo-controlled heat lamp (Yellow Springs Instruments, Yellow Springs, OH, USA) and a thermoregulated table. A tracheostomy was performed to allow spontaneous breathing. Catheters were placed in the jugular vein for infusions (inulin and saline), carotid artery for blood pressure measurements and blood sampling, and bladder for urine collection.

Determination of the maximum capacity for phosphate reabsorption in sham and UNX rats

Following general surgical preparation, animals from groups 1 and 3 underwent thyroparathyroidectomy (TPTX) to remove the influence of endogenous parathyroid hormone (number of animals in these groups given under prior group description). Following TPTX, a 2% cold inulin (Sigma Chemical Co., St. Louis, MO, USA)/saline solution was infused at a rate of 2% body wt/hr. After a 90-minute recovery period, a 20-minute control urine clearance was made, with a blood sample taken at the midpoint of the collection. To increase the filtered load of Pi to the kidneys and facilitate determination of the TmPi, increasing concentrations of phosphate (di- and mono-basic in a 4:1 ratio) sodium phosphate dissolved in normal saline) were added to the inulin solution every hour (to achieve rates of 3, 6, and 9 μmol/min). Urine clearances were taken continuously every 20 minutes for an additional 100 minutes, and blood samples were obtained at the midpoint of each clearance. At the end of the experiment, the remnant kidney or both sham kidneys were weighed and placed in 10% formalin for morphological analysis. TmPi was calculated as the mean of the highest individual values of reabsorbed Pi per ml glomerular filtration rate (RPi/GFR) in each group [18].

Renal response to saline volume expansion in sham and UNX rats

During general surgical preparation described previously, animals in group 2 had an additional catheter containing saline inserted in the same jugular vein as the inulin catheter (number of animals in these groups given under prior group description). The syringe pump controlling the saline catheter was not turned on during the 60-minute recovery period. For these experiments, the thyroid and parathyroid glands remained intact.

After the 60-minute recovery period, two 20-minute control urine clearances were obtained, with blood samples taken at the midpoint of each clearance. The saline infusion was started at an additional 2% body wt/hr for one hour and then 4% body wt/hr for one hour. Urine clearances and blood samples were made every 30 minutes over the experimental period. Steady-state levels were assessed during the second 30-minute clearance of each saline infusion, and these values are reported. At the end of the experiment, animals were killed. The kidney(s) was weighed, bisected, and placed in 10% formalin for morphological analysis.

Morphometric studies

To assess the potential gender-related differences in kidney tissues initiated by UNX, morphological studies were performed on control and remnant kidneys from adult female and male rats from groups 1, 2, and 3 two-months post-UNX. All kidneys were coded and analyzed in a blind study. The code was keyed after all samples were analyzed. Two micrometer sections of formalin-fixed methacrylate-embedded tissue from longitudinal slabs of kidney were stained with periodic acid-Schiff reaction. The areas of at least 100 glomerular tuft profiles per sample were measured interactively with an *ad hoc* routine running Optimas© image analysis system (Bioscan, Edmunds, WA, USA.) An estimate of the mean glomerular volume (MGV) was derived from the harmonic mean of unselected profile areas, as previously described [19, 20].

Metabolic studies

Metabolic studies were performed in female sham-operated ($N = 3$) and UNX rats (UNX only, $N = 7$; +Ox/VEH, $N = 5$; +Ox/EST, $N = 5$; +Ox/TEST, $N = 5$) at two-months postsurgery to assess the effects of changes in the TmPi on Pi excretion in the conscious animals. At two-months postsurgery, animals were placed in individual metabolic cages (Nalgene) three days prior to performing acute TmPi studies. Food and water intake and urine and fecal output were measured daily, and urine and fecal samples were collected for analysis of phosphate and sodium content. Electrolyte balance was determined as the difference between intake (in food) and urine and fecal output.

Analysis

Glomerular filtration rate was equated with the clearance of cold inulin by the Anthrone colorimetric method [21]. Urine and plasma sodium and potassium concentrations were measured by flame photometry (IL-943; Instrumentation Laboratories, Lexington, MA, USA). Phosphate concentrations in urine and plasma were determined by the phosphomolybdate method of Chen, Toribara, and Warner [22]. Fecal samples were washed at 600°F in a muffle furnace and then ground and resuspended in 2 ml of 2 N HCl for analysis, as previously described [18].

Statistical analysis

Comparisons between kidneys from female and male animals were made using unpaired Student's *t*-tests. Comparisons between control and remnant kidneys within the same sex animal were made using paired Student's *t*-tests. Linear regression was performed with Pearson's method. A significance of percentage changes in glomerular volume was performed using the Mann–Whitney U nonparametric analysis. Significance was assigned as $P < 0.05$.

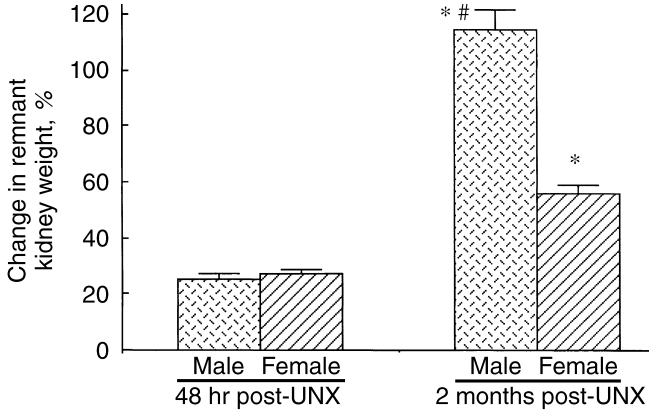

Fig. 1. Percentage change in remnant kidney weight (KW) 48-hours and two-months post-UNX in adult male and female rats. Although initial remnant kidney growth was not different between the sexes, male remnant kidneys grew a significantly greater extent than female remnant kidneys. *$P <$ at least 0.05 vs. control kidney; #$P <$ of at least 0.05 vs. female remnant kidney.

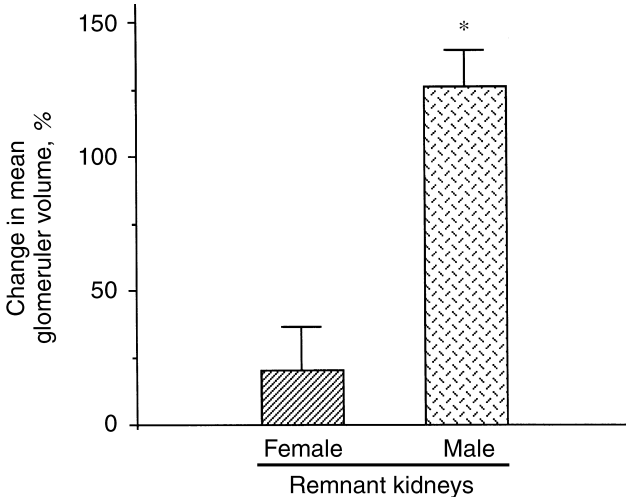

Fig. 2. Percent change in mean glomerular volume (MGV) in male and female remnant kidneys at two-months post-uninephrectomy (UNX). MGV was not changed in female kidneys compared with sham-operated controls; however, there was a significant increase in MGV in remnant kidneys from male UNX rats. *$P < 0.001$ vs. female MGV.

RESULTS

Remnant kidney growth and morphology after UNX

Remnant kidney growth was determined at two days (group 4) and two months post-UNX (groups 1 through 3), and potential pathological changes were assessed at two months following UNX or sham operation (animals were then 5 to 6 months of age). The remnant kidney was compared with the excised kidney from each individual animal. Figure 1 illustrates that although the initial (48-hr post-UNX) increase in remnant kidney weight (KW) was not different between the sexes, growth was doubled in the male compared with the female remnant kidneys after two months (Δ in remnant KW of 114 $\pm$ 7 vs. 57 $\pm$ 5%, respectively, $P < 0.001$). Although the female remnant kidneys did not grow to the same extent as observed in the male, they weighed significantly more than a single age- and weight-matched female control kidney (KW/body wt proportionality ratios of 0.46 $\pm$ 0.01 vs. 0.31 $\pm$ 0.01% in female remnant and control kidneys, respectively, $P < 0.0001$). As to be expected, the difference in KW/body wt ratios was considerably greater in the male remnant compared with both control kidneys (0.59 $\pm$ 0.02 vs. 0.39 $\pm$ 0.01%, respectively, $P < 0.0001$), despite the greater increase in weight gain in the males. Over the two-month period, body weights increased 43 $\pm$ 5 g in female, and 156 $\pm$ 7 g in male rats ($P < 0.0001$). The KW/body wt ratios for male control and remnant kidneys were significantly greater ($P < 0.01$) than the corresponding values for female kidneys.

Morphometric analysis of control and remnant kidneys of male and female rats exposed additional gender differences. MGV was significantly greater in control kidneys from female rats compared with that in male rats (1.89 10^6 μm^3 vs. 0.85 10^6 μm^3, $P < 0.001$). Although there was no significant change in MGV at two-months post-UNX in the female remnant kidneys (Δ20.2 $\pm$ 16.1%), there was a dramatic increase in MGV in male remnant kidneys (Δ126.2 $\pm$ 13.4%, $P < 0.0005$ from control values; Fig. 2). In addition, the increase in glomerular volume was proportional to the original size of the contralateral, control male kidney (Pearson's linear regression, $r^2 = 0.66$, $P < 0.05$). Furthermore, this increase in glomerular size was associated with other pathological changes in the male remnant kidneys. There were no glomerular or tubular anomalies in the control kidneys of either sex (Table 1). Female remnant kidneys had very slight tubular dilation and tubular cell swelling, a finding that is in keeping with a small, long-term hypertrophy of the tubular cells commensurate with the CRG. In sharp contrast to the findings in the female kidneys, male remnant kidneys had significant focal hydronephrosis with whole nephron damage, including tubular dilation, tubular cell swelling, Bowman's space enlargement, and glomerular tuft compression. Two out of six remnant kidneys also contained patchy lymphoid infiltrates (Table 1).

The effects of gonadal steroid replacement on remnant kidney growth was determined in Ox female rats. As expected, Ox/TEST rats displayed a greater body weight gain over the two-month period following UNX compared with Ox/EST or Ox/VEH animals (95 $\pm$ 9 vs. 54 $\pm$ 7 and 58.0 $\pm$ 5 g, respectively, $P < 0.02$). Although our Ox/TEST treatment regimen did not stimulate the same degree of glomerular and tubular damage observed in intact male UNX remnant kidneys, there was a dramatic increase in glomerular volume in the Ox/TEST remnant

Table 1. Renal pathology in remnant kidneys post-uninephrectomy

	Renal lesions		
	Single glomerular hydronephropathy	Tubular dilation tubular cell swelling	Lymphoid infiltrates
Female			
Control ($N = 4$)	–	–	–
Remnant ($N = 6$)	–	(±) (6/6)	–
Male			
Control ($N = 6$)	–	–	–
Remnant ($N = 6$)	+ (5/6)	+ (6/6)	+ (2/6)

The abbreviation *N* is the number of kidneys; (#pos/total).

Table 2. Basal parameters in sham-operated and unenephrectomized (UNX)

	Female		Male	
	Sham ($N = 9$)	UNX ($N = 18$)	Sham ($N = 10$)	UNX ($N = 13$)
GFR *ml/min*	2.59 ± 0.12	2.29 ± 0.17	3.45 ± 0.30	2.88 ± 0.23[b]
$U_{Na}V$ *μEq/min*	0.49 ± 0.27	5.24 ± 1.45[a]	0.68 ± 0.31	10.41 ± 1.88[ab]
FE_{Na} %	0.18 ± 0.05	1.19 ± 0.22[a]	0.17 ± 0.04	2.0 ± 0.04[ab]
FE_K %	22.5 ± 3.2	39.2 ± 4.1[a]	30.0 ± 3.5	29.9 ± 2.3[b]
FE_{Pi} (TPTX) %	0.01 ± 0.01	4.67 ± 1.29[a]	0.01 ± 0.03	0.90 ± 0.80[b]
Plasma Na *mEq*	140 ± 3	140 ± 2	147 ± 1	148 ± 1[b]
Plasma K *mEq*	3.4 ± 0.1	3.2 ± 0.1	3.8 ± 0.1	3.9 ± 0.1[b]
Plasma Pi *mM*	1.92 ± 0.08	1.56 ± 0.20[a]	2.23 ± 0.23	1.99 ± 0.05[b]
$\dot{V}$ *μl/min*	12.2 ± 5.4	27.6 ± 6.1[a]	11.5 ± 1.5	71.3 ± 11.1[ab]

Abbreviations are: GFR, glomerular filtration rate; $U_{Na}V$, urinary sodium excretion; FE_{Na}, fractional excretion of sodium; FE_{Pi}, fractional excretion of phosphate (Pi); FE_K, fractional excretion of potassium; TPTX, thyroparathyroidectomy; $\dot{V}$, urine flow rate.

[a] P of at least < 0.05 vs. sham control value

[b] P of at least < 0.05 vs. female UNX

kidneys, which was significantly greater than that observed in ovary intact remnant kidneys (53 ± 6 vs. 14 ± 4%, respectively, $P < 0.003$).

Basal renal function at two-months post-UNX

Basal renal function was determined in sham and UNX male and female rats at two months post-UNX. When animals from groups 1 and 2 were prepared for their respective acute renal clearance experiments, basal parameters were assessed in the control period, prior to initiation of the experimental protocols. Remnant kidney function was increased in both female and male rats two months following UNX, commensurate with the gain in remnant KW. As expected, the remnant GFR/KW ratios in female rats were significantly greater than the GFR/KW ratios in control two-kidney female animals (1.31 ± 0.11 vs. 0.59 ± 0.07 ml/min/g KW, respectively, $P < 0.02$). This indicates that the enlarged single kidney markedly increased the GFR over the two-month period. This also held true for the UNX male animals (GFR/KW, 1.33 ± 0.12 vs. 0.72 ± 0.11 ml/min/g KW, respectively, $P < 0.01$). Interestingly, basal urinary ($U_{Na}V$) and fractional (FE_{Na}) sodium excretion was elevated in UNX female and male rats. In addition, $U_{Na}V$ and FE_{Na} were significantly greater in the male UNX rats compared with females (Table 2). Urine flow rate was also significantly higher in the UNX animals, possibly because of the increase in sodium excretion. Interestingly, the fractional excretion of phosphate (FE_{Pi}) was significantly higher than controls in UNX female, but not UNX male rats. This may have been reflected in the somewhat low plasma phosphate levels in the female UNX rats (Table 1). Plasma sodium and potassium levels were not different than sham-operated controls within the sexes; however, the female rats had consistently lower values than the males in this paradigm (Table 2).

Basal parameters for female OX rats supplemented with steroids are given in Table 3. The GFR was not significantly different between groups, although there was a tendency for the Ox/TEST animals to have higher rates. Both the EST- and TEST-replaced Ox rats had significantly increased basal fractional excretion of sodium, as observed in the intact male and female UNX rats. Ox/TEST, but not Ox/EST, animals displayed a significantly greater urine flow rate than VEH-treated animals, possibly because of the greater sodium excretion. Fractional potassium excretion was also greater in the Ox/TEST animals. The differential effects of TEST and EST were especially evident in the fractional excretion of Pi. Consistent with findings in the UNX male and female rats, FE_{Pi} was significantly elevated in the Ox/EST rats compared with VEH- and TEST-treated animals

Table 3. Basal parameters in ovariectomized UNX rats

	Ox/VEH ($N = 5$)	Ox/EST ($N = 5$)	Ox/TEST ($N = 5$)
GFR *ml/min*	2.28 ± 0.27	1.85 ± 0.28	2.68 ± 0.59
FE_{Na} %	0.40 ± 0.20	1.15 ± 0.21[a]	1.64 ± 0.49[a]
FE_K %	18.2 ± 3.7	17.7 ± 5.7	41.6 ± 7.2
FE_{Pi} (TPTX) %	0.40 ± 0.30[b]	5.45 ± 2.13[a]	0.66 ± 0.14[b]
Plasma Pi *mM*	1.82 ± 0.17	2.10 ± 0.10	2.39 ± 0.05[a]
$\dot{V}$ *μ/min*	19.3 ± 8.5	18.9 ± 2.1	38.5 ± 10.6[ab]

Abbreviations are: GFR, glomerular filtration rate; $U_{Na}V$, urinary sodium excretion; FE_K, fractional excretion of potassium; FE_{Na}, fractional excretion of sodium; FE_{Pi}, fractional excretion of phosphate (Pi); TPTX, thyroparathyroidectomy; $\dot{V}$, urine flow rate.

[a] P of at least < 0.05 vs. Ox/VEH

[b] P of at least < 0.05 vs. Ox/EST

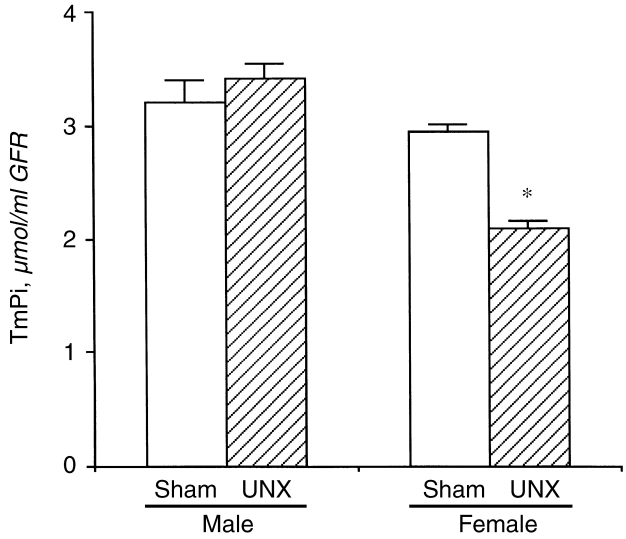

Fig. 3. Maximum capacity for phosphate reabsorption (TmPi) in adult male and female rats at two-months postoperation. TmPi (determined after TPTX) was not different following UNX in male rats; however, there was a significant reduction in the capacity to reabsorb Pi in the UNX female rats. *$P < 0.001$ vs. sham-operated rats.

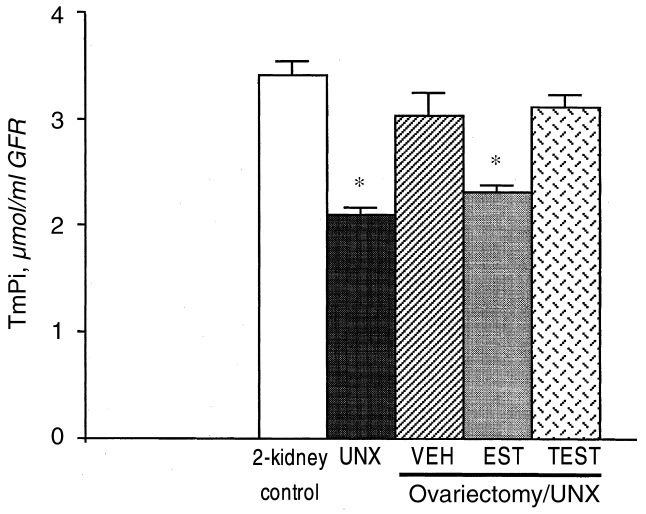

Fig. 4. Maximum capacity for phosphate reabsorption (TmPi) in female rats at two-months postoperation. TmPi (determined after TPTX) was not different from two-kidney (sham-operated) controls in OX/VEH and Ox/TEST UNX rats; however, there was a significant reduction in the capacity to reabsorb Pi in the Ox/EST female rats, which was similar to that observed in ovary-intact UNX female rats. *$P < 0.001$ vs. sham-operated rats.

(Table 3). This supports the concept that EST promotes Pi excretion in the female UNX animal.

Maximum capacity for phosphate reabsorption in sham and UNX rats

To determine whether the increased basal FE_{Pi} in female UNX rats was associated with a decrease in the intrinsic renal tubular capacity for phosphate reabsorption, TmPi studies were performed. Basal FE_{Pi} (Table 2) and TmPi (Fig. 3) in male rats were not different from sham-operated control animals, indicating that Pi handling was not affected by a loss of renal mass after two-months post-UNX. In contrast, there was a marked decrease in the TmPi (Δ –41% from controls) observed in female UNX rats (Fig. 3).

Consistent with the difference in Pi handling observed in intact male and female UNX rats, TmPi in Ox/VEH and Ox/TEST animals was not different from female sham-operated controls or intact UNX male rats. Moreover, the Ox/EST rats displayed a significant reduction in TmPi, which was not different from intact female UNX rats (Fig. 4). Also, basal FE_{Pi} was significantly increased in the Ox/EST compared with VEH- or TEST-treated animals (Table 3). This confirms the increase in FE_{Pi} observed in intact female UNX animals (Table 2) and indicates that EST does cause an increase in Pi excretion in the UNX female.

Renal response to saline volume expansion in sham and UNX rats

The acute renal response to saline volume expansion was also determined in anesthetized animals two months after sham operation or UNX (group 2). Again, basal urinary sodium excretion and urine flow were elevated in both female and male UNX rats (Table 2). Figure 5 illustrates that in response to 2 and 4% saline volume expansion, urinary sodium excretion was significantly

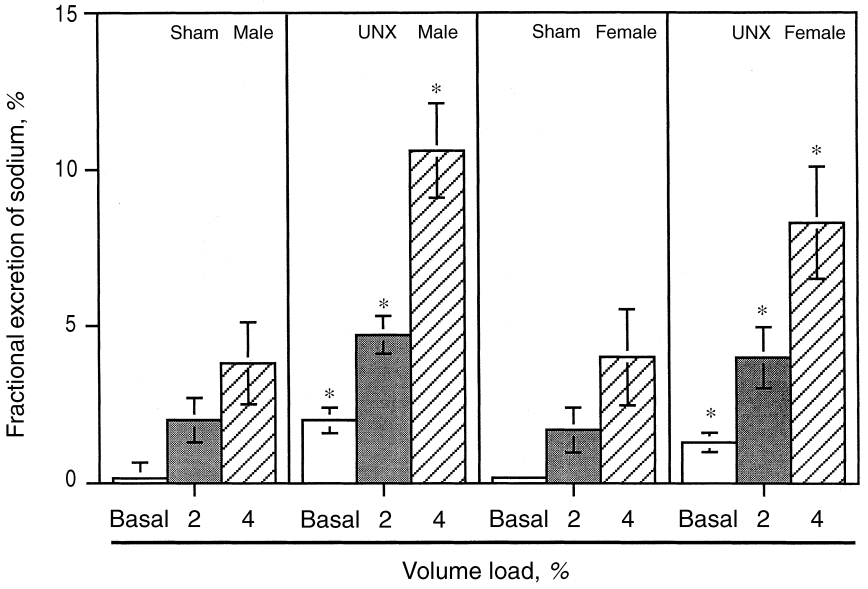

Fig. 5. Fractional excretion of sodium (FE_{Na}) following 2 and 4% saline volume expansion (VE) in male and female rats two months following UNX or sham operation. Basal FE_{Na} was elevated above control levels in both male and female UNX rats. In response to VE, UNX animals exhibited an exaggerated natriuresis compared with their sham-operated controls. *$P <$ at least 0.05 vs. control time point. B, basal.

higher in UNX rats compared with their sham-operated controls. The fractional excretion of sodium was not different between female and male UNX rats at any time point. Urine flow rate was also elevated above sham controls during volume expansion periods.

Metabolic studies in conscious female rats

To address the potential effects of attenuated phosphate reabsorption observed in the UNX female rat, metabolic studies were performed in a separate group of conscious female rats (2 months post-UNX or sham operation) prior to acute TPTX and determination of TmPi (groups 1 and 3). Over the collection period, UNX females tended to lose weight more than that observed in sham-operated controls (–10.3 ± 3.5 vs. –3.0 ± 0.5 g over the two day experimental period, $P = 0.1$), although the difference was not significant. Food and water intake was comparable in control and UNX rats over the three day period. However, although water intake was not different over the three day period (37.0 ± 0.7 vs. 37.4 ± 3.9 ml/day in UNX and control animals), urine volume was significantly elevated over control adult rats (21 ± 3 vs. 9 ± 1 ml/day, respectively, $P < 0.05$). Fecal weight was reduced in UNX rats compared with controls (8.0 ± 0.8 vs. 14.6 ± 1.1 g/day, respectively, $P < 0.01$). Urinary phosphate excretion was significantly increased two months post-UNX compared with controls (0.72 ± 0.06 vs. 0.49 ± 0.03 mmol/day, $P < 0.03$). This was consistent with the reduced TmPi observed in the acute clearance studies. The normally high positive phosphate balance observed in the two-kidney female animals (58 ± 2%) was markedly decreased in the UNX female (to 28 ± 3%, $P < 0.0001$). Urinary sodium excretion was also elevated in UNX female rats (1.95 ± 0.14 vs. 1.45 ± 0.08 mEq/day in control rats, $P < 0.04$), and the combined urinary and fecal sodium excretion reduced sodium balance in the adult female animals by 50% (from 46 ± 4% in controls to 20 ± 10% in UNX rats, $P < 0.05$), which is again consistent with the basal sodium excretion in the acute clearance studies.

The effects of gonadal steroids on metabolic parameters was assessed in conscious, steroid-supplemented Ox rats. The average daily food intake over the three days was similar between control females (no UNX) and Ox rats treated with EST or TEST (17.8 ± 0.5, 18.5 ± 0.8, and 14.7 ± 1.1 g/day, respectively). Water intake was significantly ($P < 0.05$) greater in Ox/EST rats (45.4 ± 1.7 ml/day) than Ox/TEST or control animals (36.4 ± 3.3 and 37.0 ± 0.7 ml/day), although urine volume was elevated in both Ox/EST (17.9 ± 1.0 ml/day) and Ox/TEST (17.3 ± 3.1 ml/day) compared with controls (9.0 ± 1.1 ml/day, $P < 0.01$). Interestingly, fecal weight was reduced in EST- and TEST-treated animals compared with control animals (9.1 ± 0.9 and 6.7 ± 0.9 vs. 14.6 ± 1.1 g/day, respectively, $P < 0.01$), suggesting altered intestinal water absorption (because food intake was comparable). Most importantly, the key differences observed in urinary phosphate handling and balance were confirmed, in that Ox/EST animals displayed significantly greater urinary Pi excretion (0.810 ± 0.12 mmol/day, $P < 0.01$) than Ox/TEST or control rats (0.48 ± 0.04 and 0.49 ± 0.03 mmol/day, respectively). This enhanced Pi excretion in the EST-treated rats reduced Pi balance down to 32 ± 5% ($P < 0.01$) from levels observed in TEST (56 ± 4%) and control (58 ± 2%) animals. These data confirm that the presence of EST, but not TEST, in the UNX rat is

associated with elevated Pi excretion. The metabolic and renal function studies indicate that UNX is associated with elevated fluid and electrolyte excretion, and in the female, the urinary losses of phosphate most likely result from a reduction in the capacity to reabsorb phosphate.

DISCUSSION

The findings of this study highlight the gender differences in remnant kidney growth, morphology, and function in rats two months after uninephrectomy. Although initial (48 hr) accelerated remnant kidney growth was comparable between the sexes, long-term (two month) kidney growth was markedly greater in the male rat. This, however, was associated with significant glomerular hypertrophy and glomerular and tubular lesions in the male remnant kidneys, which were associated with the presence of TEST. In contrast, remnant kidneys from female rats had no relevant morphological lesions. The renal growth was associated with the hallmark compensatory increase in GFR in both genders. Of additional interest were the findings that renal sodium and phosphate handling are significantly altered even at this fairly early period post-UNX. Metabolic studies determined that there was an EST-dependent increase in urinary Pi excretion in conscious female UNX rats, which was consistent with the acute clearance data. The acute clearance studies also determined that, indeed, basal urinary sodium and fluid excretion was elevated in both male and female anesthetized rats. An important distinction between the sexes was that, although UNX male rats maintained a normal capacity to reabsorb phosphate, the female UNX rats had a 40% reduction in TmPi, which was EST dependent, and resulted in the attenuated phosphate balance observed in conscious female rats. In summary, these findings suggest that there are important gender differences in the CRG response, which include early glomerular hypertrophy and damage in the male, a reduction in phosphate handling in the female, and tendency for altered sodium handling in both sexes.

Although many studies have been published on human kidney donors, invasive testing cannot be performed in the clinical setting, and many renal functional and morphological parameters were assessed only when overt disease was present. Previous studies in humans and animals have reported the presence of gender differences in renal growth and function after nephrectomy. It has been shown that men with a single kidney may have increased proteinuria and hematuria compared with women [7–10], a finding also observed in animals [14]. Baylis reported that the glomerular damage observed in the aging male rat kidney is dependent on the presence of TEST, although the damage in the male was found to be dissociated from glomerular hypertrophy and hypertension [15]. Although this is true with normal aging in the male, our studies suggest that UNX accelerates the process, and in this pathophysiological case, the hypertrophy may be associated with accelerated, early glomerular, and tubular damage in the male remnant kidney. This effect of TEST has also been observed after a longer period of time post-UNX in male rats, and castration attenuated the glomerular injury and hypertrophy [23]. In that study, EST supplementation did not reduce glomerular hypertrophy; however, the steroid was given to gonad-intact animals, so the TEST was still present. Our future studies will investigate the relationship between factors that stimulate the glomerular hypertrophy and those that contribute to early renal damage.

One of the intriguing findings was the increase in Pi excretion observed in the female, but not male UNX animals. The steroid replacement studies indicate that the effect on Pi handling was EST dependent. The significant increase in Pi excretion observed in ovary intact and Ox/EST female animals in the metabolic studies is completely consistent with the reduction in TmPi in these animals. The TmPi normally is significantly higher than the plasma Pi level, insuring that adequate Pi will be reabsorbed. In the EST-replete UNX female rats, the TmPi was reduced to levels at or near the plasma level of Pi [even in the absence of parathyroid hormone (PTH)], which allowed the excretion of significant amounts of Pi in both the acutely TPTX and conscious PTH-replete animals. This is a dramatic effect, and considering the importance of proper Pi homeostasis to various metabolic processes, including bone remodeling, this finding may have important implications. This modest Pi loss observed in this study may not overtly compromise metabolic processes under normal circumstances, but during periods of increased demand for Pi, such as during pregnancy or for bone remodeling, it is unclear whether the kidney could adapt to the demand. There is the possibility that a pernicious disease state may ensue. Recent findings suggest that EST actually inhibits sodium-dependent Pi transport in the kidney [24]. This action of EST may be exacerbated in the single-kidney female animal, as observed in this study. If Pi handling is similar in human single-kidney females, postmenopausal EST replacement may actually cause an imbalance in the plasma calcium-phosphate ratio necessary for maintenance of proper bone mineralization. The impact of altered Pi handling in females warrants further study.

Although Pi handling was altered only in the female (EST-replete) UNX animals, sodium excretion was affected in both male and female UNX rats. This was evident from the enhanced basal FE_{Na}, as well as in the exaggerated natriuretic and diuretic response to saline volume expansion compared with sham-operated controls. This was also confirmed in the OX rats treated with TEST and EST (Table 3). Although water intake

was only elevated in the Ox/EST animal, both EST- and TEST-treated animals had significantly reduced fecal weight, suggesting that intestinal absorption of water may be stimulated. Indeed, although plasma sodium and potassium levels are not different following UNX (Table 2), the chronic sodium loss may be stimulating aldosterone secretion, which can act on the colon to dehydrate the feces. This additional fluid absorption may balance out the urinary losses associated with the natriuresis.

It is unknown whether circulating PTH levels are altered in the female UNX animal or whether they are different from those observed in the male. Considering that PTH is one of the primary regulators of Pi homeostasis, it is possible that it is elevated to a greater extent in the female UNX animal than the male; however, because the TmPi is calculated in the absence of PTH, the decreased TmPi observed in the UNX female and Ox/EST rats appears to be a PTH-independent, intrinsic change. Adding the phosphaturic influence of PTH in the conscious animals augments the Pi excretion, as seen in the metabolic studies.

This study indicates that gonadal steroids play a role in determining the mechanisms for the initial growth response, as well as the long-term growth and changes in renal function. Gonadal steroid receptors are present in the kidney [25–28], and although differential regulation of the EST receptor is observed in various target organs, the effects of EST and TEST on the normal and remnant kidney are not well documented. However, the earlier work of Reckelhoff and Baylis provides valuable insight into the pathological role of TEST in the remnant kidney [15, 16]. It is relevant to note that these studies were performed nine-months post-UNX, when overt pathology was present. Our studies have expanded on this work by determining that glomerular hypertrophy occurs at a relatively early time (two months) after UNX and is well established when early glomerular and tubular lesions are beginning to occur. The observation that glomerular hypertrophy occurred in the male and Ox/TEST remnant kidneys highlights the role of TEST in the pathological process following UNX.

Our working hypothesis is that the presence of TEST drives the growth-hormone–related mechanism occurring after UNX. When TEST is present, early CRG is stimulated by GH to produce a rapid, hypertrophic growth, potentially with activation of a myriad of other cofactors after the initial surge of GH. Stimulating this GH-dependent growth may also significantly contribute to the development of glomerular hypertrophy and lesions. Recent studies have extended the early CRG mechanistic findings by determining that there is a significant increase in glomerular angiotensin (Ang) II AT1 receptor expression 48-hour post-UNX in male but not female remnant kidneys (abstract; Mok et al, *FASEB J* 12:A331, 1998). The increase in AT1 receptor expression was blocked by suppression of GH, indicating that AT1 receptors may play a role in the increase in renal growth and function during the initial phase of CRG. The increase in AT1 receptor expression in glomeruli strongly supports the concept that the development of glomerular hypertrophy may be mediated by Ang II and further points to the regulation of AT1 receptors by circulating GH. Although many studies have suggested a relationship between circulating GH and Ang II [29–32], the regulation of the AT1 receptor by GH and the potential effect on glomerular hypertrophy are novel concepts being examined by this laboratory.

In conclusion, there are consistent gender differences in renal growth, function, and pathology occurring as early as two-months post-UNX. The appearance of overt, TEST-driven glomerular hypertrophy at a relatively early time following renal ablation provides the potential mechanism for the subsequent development of glomerular and tubular lesions in the male remnant kidney. Although there were no overt morphological changes in the female remnant kidneys, there was a significant reduction in the capacity to reabsorb Pi, which could lead to Pi losses in the female, but not male animal. These findings expand on the previous gender differences observed in initial CRG and support the concept that the gonadal steroids play important roles in the differential mechanisms stimulated following renal ablation.

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